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Assessment of *Streptomyces* sp. and *Bacillus* sp. Against *Phytophthora palmivora*, the Causal Agent of Durian Canker Disease through Different Application Methods at The Nursery Stage

Nor Dalila Nor Danial[a,*], Stella Matthews[b], Nurul Fahima Mohamad Amaran[a] and Nur Aisyah Anis Abd Kharim[a]

[a] Horticulture Research Centre, MARDI Sintok, 06050 Bukit Kayu Hitam, Kedah, Malaysia
[b]Agrobiodiversity and Environment Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia

***Corresponding author: daleyla@mardi.gov.my**

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ABSTRACT

Phytophthora palmivora is a natural soil-borne pathogen that infects all parts of the durian tree. It was first reported in 1939 and is still a major problem in durian cultivation. With the increased awareness of the risks of synthetic fungicides usage, the focus of this study is to determine the efficacy of *Streptomyces* sp. and *Bacillus* sp. as singular and mixed usage in controlling *P. palmivora* using drench and spray application methods at the nursery stage. Thus, we aim to identify the relationship of the variables. Microbe treatments consist of; Control, *Streptomyces* sp. (10^8 cfu/mL), *Bacillus* sp. (10^{10} cfu/mL) and *Streptomyces* sp. + *Bacillus* sp. (10^{10} cfu/mL). The application method was by drenching and spraying method. The experiment consisted of 8 treatments in 4 replications using RCBD Design. Data were computed using SAS to assess descriptive analysis, test of a normality, analysis of variance, multiple comparisons and Pearson correlation at a 95% probability level. The mixture is grouped together with treatment *Bacillus* sp. in the mean separation using Tukey's Honestly Significant (HSD). The mixture of *Streptomyces* sp. and *Bacillus* sp. was the best in controlling the disease at 19.56 mm lesion growth and 74.35% protection percentage. Here treatment *Bacillus* sp. has a lesion growth of 28.93 mm and 62.05% protection percentage. All treatments containing microbes had a significant difference compared to control treatments (76.23 mm). As for the application method; the drenching method had a significantly higher plant leaf area of 724.7 cm^2 and leaf fresh weight of 18.43 g compared to the spraying method. As for the Pearson correlation coefficient, it is seen the leaf fresh weight (*P = <.0001; r = 0.71728*) had a significantly strong positive correlation with leaf area. Further studies are needed to verify the effect of *Streptomyces* sp. and *Bacillus* sp. at the field level, where these microbes will be subjected to current climate conditions.

Keywords: *Phytophthora palmivora*, *Streptomyces* sp., *Bacillus* sp., durian, application method

INTRODUCTION

Durian (*Durio zibethinus)* is a sought-after fruit, especially in Asian countries. According to the Trade Map (UN Comtrade, 2018), Malaysia is known to be the largest consumer of durian, with a per capita consumption of 11 kg. This economically important crop valued at RM 8.4 billion is the largest cultivated crop in Malaysia with a production rate of 448,272 MT in 2021 (Department of Agriculture, 2023). Despite a wide production avenue, the durian crop is continuously under threat from durian canker disease, caused by *Phytophthora palmivora* (Drenth & Sendall, 2001).

The pathogen, *Phytophthora palmivora* is a natural soil-borne fungus that can infect all parts of the durian tree at any stages of growth. *Phytophthora palmivora*, causes stem canker at nearly all phases of durian growth and is reported to have an estimated average loss of 20-30% in Malaysia (Misman *et al.,* 2022). On affected plantations, the standard practice is the application of chemical fungicides. Fungicide usage has become increasingly restricted due to public concerns about the accumulation of toxic chemicals that may be harmful to humans and the environment. As a result, it is vital to find an alternative to control the pathogen. Biological control appears to be a promising strategy, and has been utilised to manage a variety of diseases (Cavalcanti *et al*., 2020, Kavino *et al*., 2008; Harish *et al*., 2009). Essentially, biological control of plant diseases is a non-hazardous approach that uses an organism or multiple organisms to suppress the pathogen and reduce disease (Jaiswal *et al*., 2015). In some cases, the efficacy of biocontrol is comparable to or better than chemical fungicide, as proven by Elshahawy *et al.* (2018), where *Pseudomonas fluorescens, Bacillus subtilis,* and *Bacillus pumilus* were able to control white-rot in garlic better than normal fungicide use. The use of multiple biological control agents together can be superior to individual agents in inhibiting plant disease and offer greater effectiveness and dependability to single biocontrol strains (Stockwell, V.O., *et al.,* 2011).

In a prior research, microorganisms isolated from soils were tested for biocontrol activities such as chitinase, hydrogen, protease and cyanide production, and as well as their potential to control *Phytophthora palmivora*, durian canker disease, using agar plate assays. A dual culture assay revealed that two bacterial strains controlled more than 50% of the pathogen's growth. These microbes were identified as *Streptomyces* sp. and *Bacillus* sp. (Stella and Nor Dalila, 2018). Therefore, this research was carried out to assess the efficacy of *Streptomyces* sp. and *Bacillus* sp. as singular and mixed usages in controlling *P. palmivora* using the drench and spray application methods at the nursery stage. The second objective is to identify the relationships between the variables.

MATERIALS AND METHODS

Biological control agents

Antagonistic bacterial strains tested by Stella and Nor Dalila in 2018, *Streptomyces* sp. and *Bacillus* sp., were used in this experiment. Culture strain *Streptomyces* sp. was designated A11 and *Bacillus* sp. was designated as ACTB13 were obtained from culture collection from the Strategic Resource Research Centre, MARDI Headquarters, Serdang, Selangor.

Pathogen

Phytophthora palmivora, the pathogen causing durian canker, was isolated from infected durian bark at the Malaysian Agriculture Research and Development Institute (MARDI) in Sintok, Kedah. Ten diseased tissue samples were washed and dipped in 10% Clorox for 10 minutes. Subsequently, the tissue was re-washed with sterile distilled water three times and later air dried. The sample was then placed on V8 juice agar. The isolates were identified by their morphological characteristics (Figure 1). *Phytophthora palmivora* that have been grown for 14 days on V8 agar was employed for this experiment.

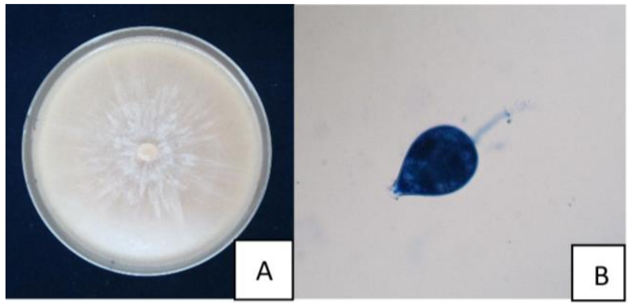

Figure 1. *Phytophtora palmivora*; (A) Culture plate on V8 juice agar at day 14 and (B) Sporangium under microscope observation (40 x)

Experimental design

Treatments consist of four microbe treatments consisting of antagonistic bacterial strains; (i) Control, (ii) *Streptomyces* sp., (iii) *Bacillus* sp., and (iv) *Streptomyces* sp. + *Bacillus* sp. Whereas the application method used were by (i) drenching and (ii) spraying. The experiment consisted of eight (8) treatments in four (4) replications. A randomized complete block design (RCBD) was used in the Plant Pathology Rainshelter in MARDI Sintok, Kedah.

All the inoculum suspensions were prepared in Nutrient Broth (Difco) in a shaking incubator at 100 rpm. *Streptomyces* sp. was incubated for 7 days and *Bacillus* sp., for 3 days. The final concentration of *Streptomyces* sp. (10^8 cfu/mL), *Bacillus* sp. (10^{10} cfu/mL), and mixture treatment of *Streptomyces* sp., + *Bacillus* sp., (10^{10} cfu/mL) was achieved before application. Treatment applications were done weekly, three (3) times before inoculation and three (3) times after inoculation. The application method was either drenching or spraying 100 mL of the inoculum suspensions mentioned. For control treatment, the inoculum suspension is substituted with sterile distilled water.

Pathogen inoculation was conducted 3 weeks following the initial application of inoculum suspension. Inoculation of the durian seedlings was conducted on the main bark. A 4 mm diameter cork borer was used to remove a small portion of the bark. A similar- sized agar plugs from the advancing margin of the fungal colony was placed onto the wounded section of the bark, then covered with a small piece of moist cotton wool, and finally wrapped with parafilm.

Parameters

Disease lesion

Measurement of the fungal infection was carried out by destructive sampling of durian seedlings on day 10. The inoculated durian seedling trunks were cut off and the bark removed to expose the lesion caused by the fungal infection. The length of the lesion was measured above and below the inoculation point. While data on the percentage of protection was calculated using the formula:

$$\text{Percentage of Protection (\%)} = \frac{R1 - R2}{R1} \times 100$$

Equation 1

Where,
R1 = Growth of pathogen alone without antagonist (Control)
R2 = Growth of pathogen along with the antagonist

Leaf area

The leaves of the durian seedlings are removed and measured using a Licor Leaf Area Meter.

Fresh weight of leaves, stem and root

The durian seedlings are cut according to plant parts; leaves, stem, and root. Each weight was then recorded using an analytical balance.

RESULTS AND DISCUSSION

Microbe

Plant diseases have a significant impact on agricultural production. The main control methods are chemical pesticides, resistant varieties and crop rotation. As of today, the common strategy is the use of pesticides. However, intensive use of chemicals leads to problems of pathogen resistance and pesticide residues (Yang *et al*., 2015). This study focused on *Streptomyces* sp. and *Bacillus* sp. in controlling patch canker disease. There have been reports that the growth of *Phytophthora* spp. can be suppressed by the use of *Streptomyces* spp. (Chen *et al*., 2016; Nguyen *et al*., 2012; Ko *et al*., 2010) and *Bacillus* spp. (Zhang *et al*., 2010).

All treatments containing microbes had a significant difference compared to the control treatments (76.23 mm) in terms of lower disease lesion growth (Table 1). The mixture of *Streptomyces* sp. and *Bacillus* sp. was the best at controlling the disease with only 19.56 mm of lesion growth and a protection percentage of 74.35%. The mixture is seen to be grouped together with treatment *Bacillus* sp. in the mean separation using Tukey's Honestly Significant (HSD) at $P < 0.05$. Treatment *Bacillus* sp. is seen to have a lesion growth of 28.93 mm and a protection percentage of 62.05%. Several compatible strains of bacterial antagonists are thought to lead to more consistent management of the disease through a more stable rhizosphere community, provide several mechanisms of biological control, and even suppress a broader range of pathogens (Boer *et al*., 2003). But in this case, there is no significant difference between the mixture of *Streptomyces* sp. and *Bacillus* sp. and the treatment using only *Bacillus* sp. In a study by Stockwell, V.O., *et al.* (2011), bacterial strain A506 was found to diminish the biological control activity of C9-1 and Eh252, lowering the effectiveness of the biocontrol mixtures. As a result, combining biological control agents does not guarantee greater disease control. Although both of these treatments were grouped together in the SAS analysis, the treatment mixture of *Streptomyces* sp. and *Bacillus* sp. gave a higher protection percentage (74.35%) than the *Bacillus* sp. treatment (62.05%).

Table 1. Main effect of antagonist microbes for disease lesion and percentage of protection (%)

Microbe	**Disease Lesion (mm)**	**Percentage of Protection (%)**
Control	$76.23^{a} \pm 24.00$	-
Streptomyces sp.	$47.10^{b} \pm 13.03$	38.22
Bacillus sp.	$28.93^{bc} \pm 6.76$	62.05
Streptomyces sp. + *Bacillus* sp.	$19.56^{c} \pm 6.83$	74.35

Application Method

Application method plays an important role in determining the right approach to the application of microbes. In this study, there was significance for parameters such as leaf area and leaf fresh weight. The drenching method was seen (Figure 2) to significantly maintain the plant leaf area at 724.7 cm^2 compared to the spraying method at 402.3 cm^2. The drenching method also had a significantly higher leaf fresh weight compared to the spray method at 18.43 g and 10.82 g, respectively (Table 2). The drenching method was able to maintain the good health of the plant, where the leaves of the durian seedlings did not wilt and fall off. Treatment of the root with a biological control agent may provide systemic protection against disease under natural environmental conditions (Ji *et al.*, 2006). Product development specifically for *Phytophthora* by drench application should be considered as all species of *Phytophthora* have a soil-borne resting stage in which its chlamydospores and oospores have a long-term survival rate in the soil (Drenth & Sendall, 2001).

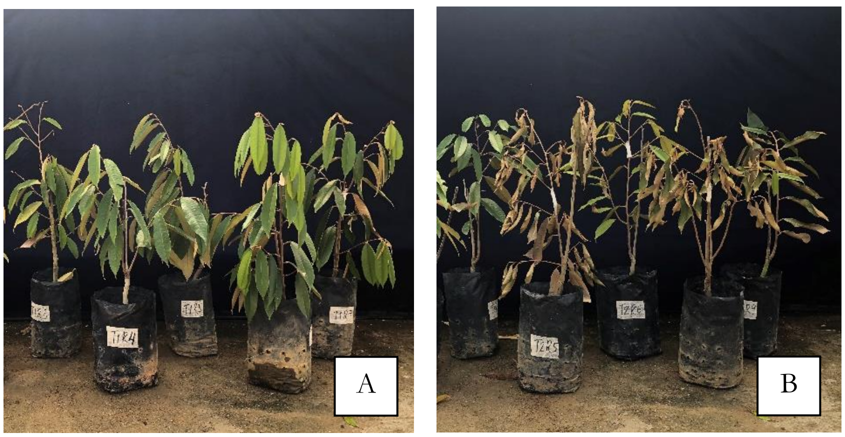

Figure 2. Effects of the application method of antagonist microbes at nursery level; (A) Drench Treatment and (B) Spray Treatment.

Table 2. Main effects of the application method for leaf area and leaf fresh weight

Application Method	**Leaf Area (cm^2)**	**Leaf Fresh Weight (g)**
Drench	724.7 ^{a} + 386.32	18.43 ^{a} + 9.24
Spray	402.3 ^{b} + 247.24	10.82 ^{b} + 9.87

As for the Pearson correlation coefficient, leaf fresh weight (r = 0.71728; P = <.0001) had a significantly strong positive correlation with leaf area (Table 3), supporting the evidence that the leaves of durian seedlings in this treatment maintained turgid. The higher the leaf fresh weight, the larger the leaf area. Contrary to a study done in 2001, the spray application method of *Pseudomonas* spp. and *Bacillus* spp. was found to be best in controlling *Phytophthora* blight on bell peppers compared to drenching (Jiang *et al.*, 2006).

Table 3. Pearson correlation coefficients of disease lesion, leaf area and leaf fresh weight

	Disease Lesion	Leaf Area	Leaf Fresh Weight
Disease Lesion	r= 1.00000	r= -0.17160	r= -0.01975
		P = 0.3477	P = 0.9146
Leaf Area		r= 1.00000	r= 0.71728
			P = <.0001*
Leaf Fresh Weight			r= 1.00000

CONCLUSION

This study demonstrated that singular use of *Bacillus* sp. and treatment containing both *Streptomyces* sp. and *Bacillus* sp. could significantly control *P. palmivora* (durian's canker) using drench application method at 62.05% and 74.35% respectively. Relationship of the treatments are identified as significantly strong positive correlation of leaf fresh weight (P = <.0001; r = 0.71728) with leaf area. Further studies will be conducted to verify the effect of *Streptomyces* sp. and *Bacillus* sp. at field conditions where the ability of these microbes to suppress the disease will be subjected to current climate conditions.

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